



## ***Impact of Herbivory and Plant Competition on the Growth of Hydrilla in Small Ponds***

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and Julie G. Nachtrieb***

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**PURPOSE:** Insect herbivory and competition have been shown to negatively impact the ability of hydrilla (*Hydrilla verticillata* (L.f.) Royle) to grow and reproduce (Doyle et al. 2002). Previous research conducted at the Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, TX and other field sites has shown that sustained levels of herbivory by the leaf-mining flies (*Hydrellia pakistanae* Deonier and *H. balciunasi* Bock) can reduce hydrilla biomass by 30 percent, reduce reproduction via reduction in flowering and tuber/turion production, and impact the ability of hydrilla to photosynthesize (Grodowitz et al. 2003; Doyle et al. 2002; Doyle et al., in review). This technical note evaluates the impacts of insect herbivory and competition on dioecious hydrilla biomass, and tuber and turion production in small ponds.

**BACKGROUND:** A native of Southeast Asia and Australia, dioecious hydrilla was first discovered in Florida in the early 1960's (Pieterse 1981). Current distribution of hydrilla in the United States can be found as far north as Maine and Washington State. Hydrilla can also be found in the Gulf and Atlantic coastal states, the western states of Arizona and California, Tennessee, and recently Arkansas (U.S. Geological Survey (USGS) 2006). Hydrilla demonstrates aggressive growth strategies, rapidly elongating to the water surface and forming a dense canopy, in addition to localized spreading through stolon growth, root crowns and tubers (Madsen and Smith 1999). The dense monotypic growth can impede navigation, destroy habitat, degrade water quality, and interfere with recreational usage.

Although there are no native insect herbivores that feed exclusively on hydrilla in the United States, two introduced leaf-mining flies have shown success at long-term management of hydrilla in controlled experimentation and field sites (Doyle et al. 2002, Grodowitz et al. 2003). The two introduced agents include the Australian leaf-mining fly (*Hydrellia balciunasi*) and the Asian leaf-mining fly (*Hydrellia pakistanae*). The larval life stages (three-instars) damage the plant by penetrating, mining and destroying hydrilla leaves (Buckingham and Grodowitz 2004). Past research (Doyle et al. 2002; Doyle et al., in review; Grodowitz et al. 2003) has shown that moderate to high levels of herbivory can impact hydrilla biomass production and reduce flowering and number of tubers produced as well as size of tubers. Doyle et al. (2002) reported that when 10-30 percent of leaves were damaged, the maximum rate of light-saturated photosynthesis was reduced by almost 40 percent. When leaf damage reached 70 percent, photosynthetic rates were reduced by up to 60 percent. This type of damage can impact hydrilla's ability to balance daily respiratory needs (Doyle et al. 2002, Grodowitz et al. 2003). Leaf damage greater than 25-35 percent can cause the hydrilla surface canopy to collapse, thus opening areas for native plant regrowth (Buckingham and Grodowitz 2004). Areas with high populations of the leaf-mining flies are often associated with a greater diversity in the plant community as reduced hydrilla growth allows other aquatic plant species to become established (Buckingham and Grodowitz 2004). Doyle et al. (in review) examined the interactions and effects of herbivory and plant competition on growth and expansion in 14,000-L mesocosm tanks over two growing seasons. Hydrilla grown in competition with

Report Documentation Page				Form Approved OMB No. 0704-0188	
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1. REPORT DATE <b>FEB 2007</b>		2. REPORT TYPE <b>N/A</b>		3. DATES COVERED <b>-</b>	
4. TITLE AND SUBTITLE <b>Impact of Herbivory and Plant Competition on the Growth of Hydrilla in Small Ponds</b>				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) <b>Engineer Research and Development Center (ERDC) 3909 Halls Ferry Rd. Vicksburg, MS 39180-6133</b>				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT <b>Approved for public release, distribution unlimited</b>					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT <b>SAR</b>	18. NUMBER OF PAGES <b>9</b>	19a. NAME OF RESPONSIBLE PERSON
a. REPORT <b>unclassified</b>	b. ABSTRACT <b>unclassified</b>	c. THIS PAGE <b>unclassified</b>			

wildcelery (*Vallisneria americana* Michx) produced less biomass and tubers. Additionally, leaf-mining herbivory negatively impacted biomass production.

The present study further examines herbivory and competition effects on dioecious hydrilla over two growing seasons in small, lined earthen ponds. This research quantifies the effects of herbivory and competition on hydrilla biomass, tuber number and size, and turion number.

**METHODS:** This study was conducted at the Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, TX (33E04'45"N, 96E57'30"W) in 21 earthen ponds. Each pond (6m x 6m with a depth of 1.3 m) was lined with 45-mil synthetic EPDM rubber (Ethylene propylene diene monomer, Firestone, Akron, OH). Unsterilized LAERF pond sediment was added to each pond to a depth of 30 cm. After leveling the sediment, LAERF pond water was added to a depth of 1.0 m.

A 2x2 factorial experimental design was used to evaluate the effects of herbivory and competition on hydrilla. The experiment utilized hydrilla in conjunction with two levels of plant competition (native plants were present or absent) and two levels of herbivory (leaf-mining flies were present or absent). To reduce the absence of leaf-mining flies in the no herbivory ponds, the insecticide ABATE 4E (Clarke Mosquito Control Products, Inc., Roselle, IL) was added at a rate of 0.41 ml per pond weekly. The ABATE was added by direct distribution into appropriate ponds.

All ponds were planted in June 2001 with 16 each mature dioecious hydrilla plants, four plants placed in each quarter of the pond. Competition ponds were randomly planted with 16 each mature wildcelery, water stargrass (*Heteranthera dubia* (Jacq.) Small), and American pondweed (*Potamogeton nodosus* Poiret). Muskgrass (*Chara* spp) and southern naiad (*Najas guadalupensis* (Sprengel) Magnus) grew from the seed bank present in the sediment. The leaf-mining flies can be found in all outdoor hydrilla ponds at the LAERF. Although small, the flies can travel short distances, moving easily between ponds. Consequently, for this study no deliberate releases were conducted and the flies were allowed to colonize naturally from the larger nearby LAERF ponds.

Leaf-mining fly populations in all ponds were quantified monthly by randomly collecting 10 hydrilla stems approximately 25 cm in length. Each stem was weighed and measured. Stems were assessed for number of damaged leaves, larvae, pupae and eggs. Leaves were considered damaged if they had evidence of leaf mining.

After two growing seasons, all ponds were drained and six replicate hydrilla samples were harvested randomly from each pond. A 0.10-m<sup>2</sup> sampling frame was placed on the sediment surface and all aboveground biomass within the frame was collected as well as sediment below the frame area. Turions were collected from the aboveground biomass and counted. The plants were sorted to species and dried at 55 °C for a minimum of 48 hr to a constant weight. The sediment was hand-washed to collect tubers. Prior to harvest, 10 stems from each pond were collected to determine numbers of immatures and associated leaf damage.

Two-Way ANOVA (Analysis of Variance) was used to determine if interactive effects occurred between plant competition (native plants) and herbivory (ABATE) using Statistica version 7 (Statsoft 2004, Tulsa, OK). F and p values are listed in the figure legends.

**RESULTS AND DISCUSSION:** Over the 2-year period of the study, the leaf-mining flies exhibited characteristic seasonal effects in the herbivory hydrilla ponds. Population numbers decreased sharply over the winter months when hydrilla was dormant. With the advent of warmer temperatures, leaf-mining fly numbers increased until cooler temperatures in the fall months stimulated hydrilla to transport nutrients and carbohydrates to the stembase, tubers, and turions for overwintering, and hydrilla aboveground biomass decreased (Madsen and Owens 1998). The non-herbivory ponds ranged in population numbers from 0 to approximately 2000 immatures/kg (Figure 1A) while the herbivory ponds ranged from 0 to greater than 5000 immatures/kg (Figure 1B). Percent damaged leaves ranged from 0 percent to greater than 50 percent during the early fall months of 2001 and 2002 for the herbivory ponds (Figure 1C) and less than 10 percent for the non-herbivory ponds (Figure 1D). Visible differences were also found between the herbivory and non-herbivory ponds. The ABATE treatments in the non-herbivory ponds suppressed larval populations and decreased overall damage to the hydrilla. The hydrilla in the non-herbivory ponds appeared greener in color and structurally intact, while hydrilla in the herbivory ponds appeared browner in color with increased filamentous algae mats forming on the surface of the ponds.

During the first growing season, native plants were observed and quantified in the competition ponds. American pondweed was observed at the surface in all of the native competition ponds ranging in coverage from 30 to 90 percent. Hydrilla was still expanding at this time and American pondweed was able to compete and produce floating leaves at the water surface. Although not intentionally planted, southern naiad and muskgrass were observed growing in the native competition ponds. Southern naiad and muskgrass, which came in via the sediments, are early spring pioneer species and were able to preempt the hydrilla expansion during the first growing season. By the second growing season, hydrilla was well established in the native plant competition ponds; therefore much less American pondweed was able to reach the surface. Additionally, in the early spring of the second year since hydrilla was well established and had overwintered within the confines of these ponds, southern naiad and muskgrass were not able to compete as the available niche was filled. In addition to hydrilla expansion within the ponds, spring filamentous algae coverage of the surface reduced light penetration and may have decreased aquatic plant survival.

Significant differences were found for aboveground biomass between the hydrilla ponds with and without herbivory (Figure 2A), although no significance was found between competition effects (Figure 2B). No interaction was noted (Figures 2C and 2D). Non-herbivory ponds averaged greater than 260 g DW m<sup>-2</sup> aboveground biomass when compared to ponds with sustained herbivory that averaged only about 150 g DW m<sup>-2</sup> aboveground biomass. High levels of herbivory by the leaf-mining flies have been reported to reduce hydrilla biomass by approximately 30 percent (Doyle et al. 2002). This study found a greater than 42 percent reduction in biomass between non-herbivory and herbivory ponds.

Significant differences were observed for hydrilla turion numbers, which were lower in the hydrilla herbivory ponds (Figure 3A) versus the non-herbivory ponds (Figure 3B). Approximately twofold more turions were recorded in the non-herbivory ponds compared to the herbivory ponds. Turions are formed in the axils of branches or leaves (Langeland 1999, Netherland 1999) so it is logical turions are more susceptible to leaf-mining fly feeding. No significant differences were observed for competition (Figure 3B) or the interaction between herbivory and competition (Figures 3C and 3D).

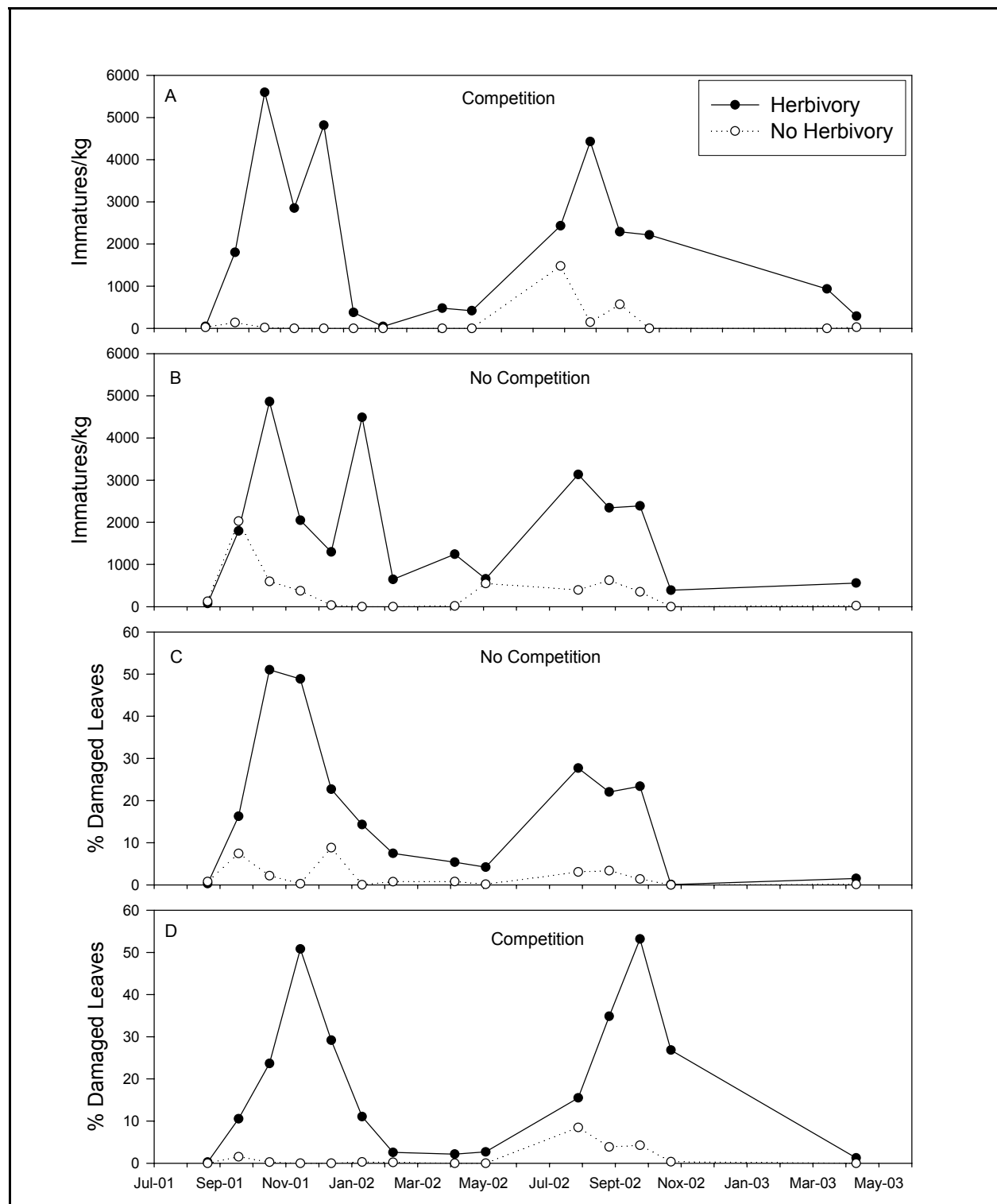


Figure 1. Number of immatures per kg in hydrilla herbivory and no herbivory ponds with competition (Figure 1A) and without competition (Figure 1B) from native plants and percent damaged leaves in hydrilla herbivory and no herbivory ponds with competition (Figure 1C) and without competition (Figure 1D).

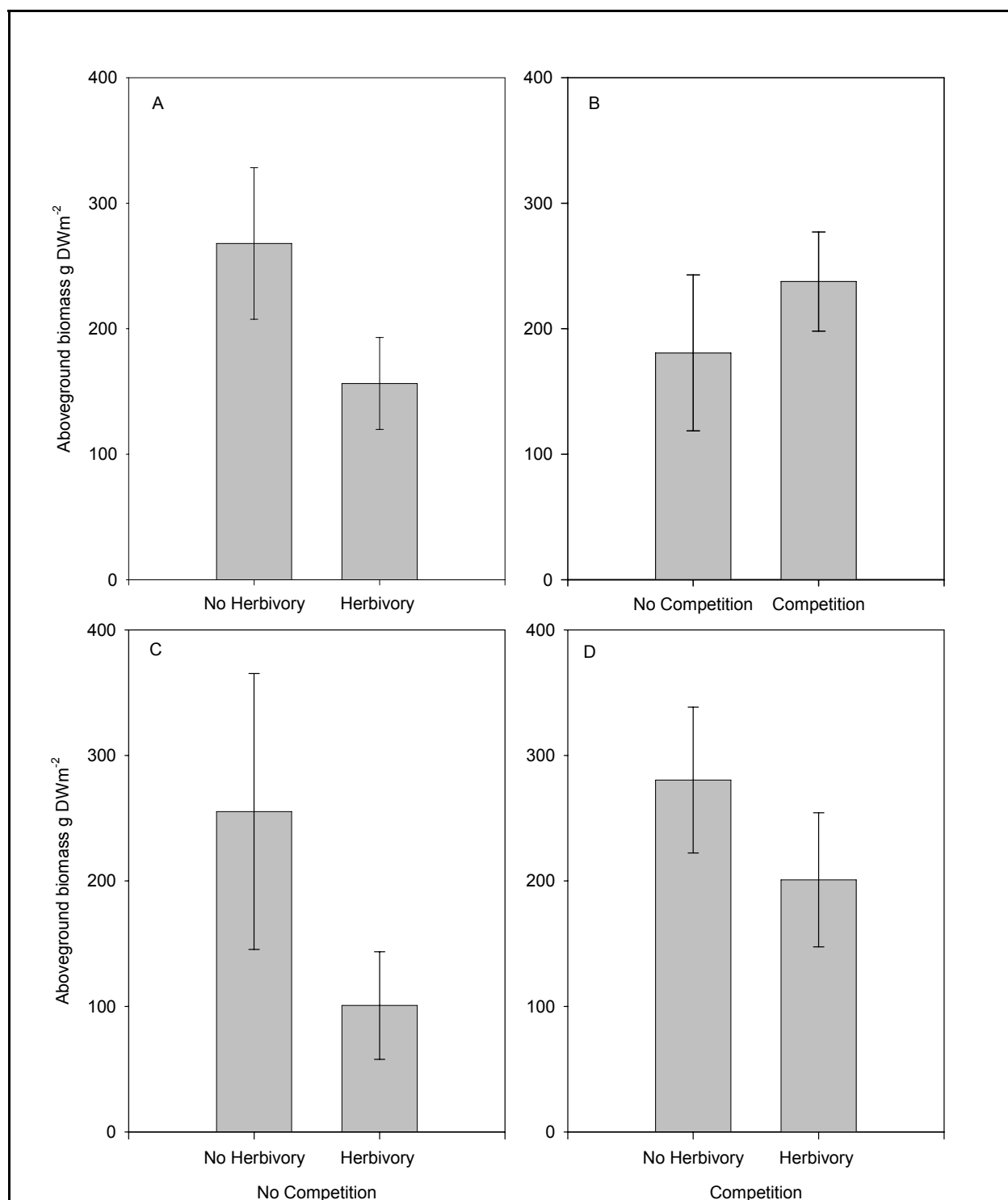


Figure 2. Aboveground biomass of hydrilla from ponds with and without herbivory ( $p=.00099$ ,  $F=11.4$ , Figure 2A) with and without competition ( $p=.07361$ ,  $F=3.26$ , Figure 2B) and hydrilla interaction between all treatments ( $p=.28114$ ,  $F=1.17$ , Figure 2C,D). Bars represent 95-percent confidence intervals.

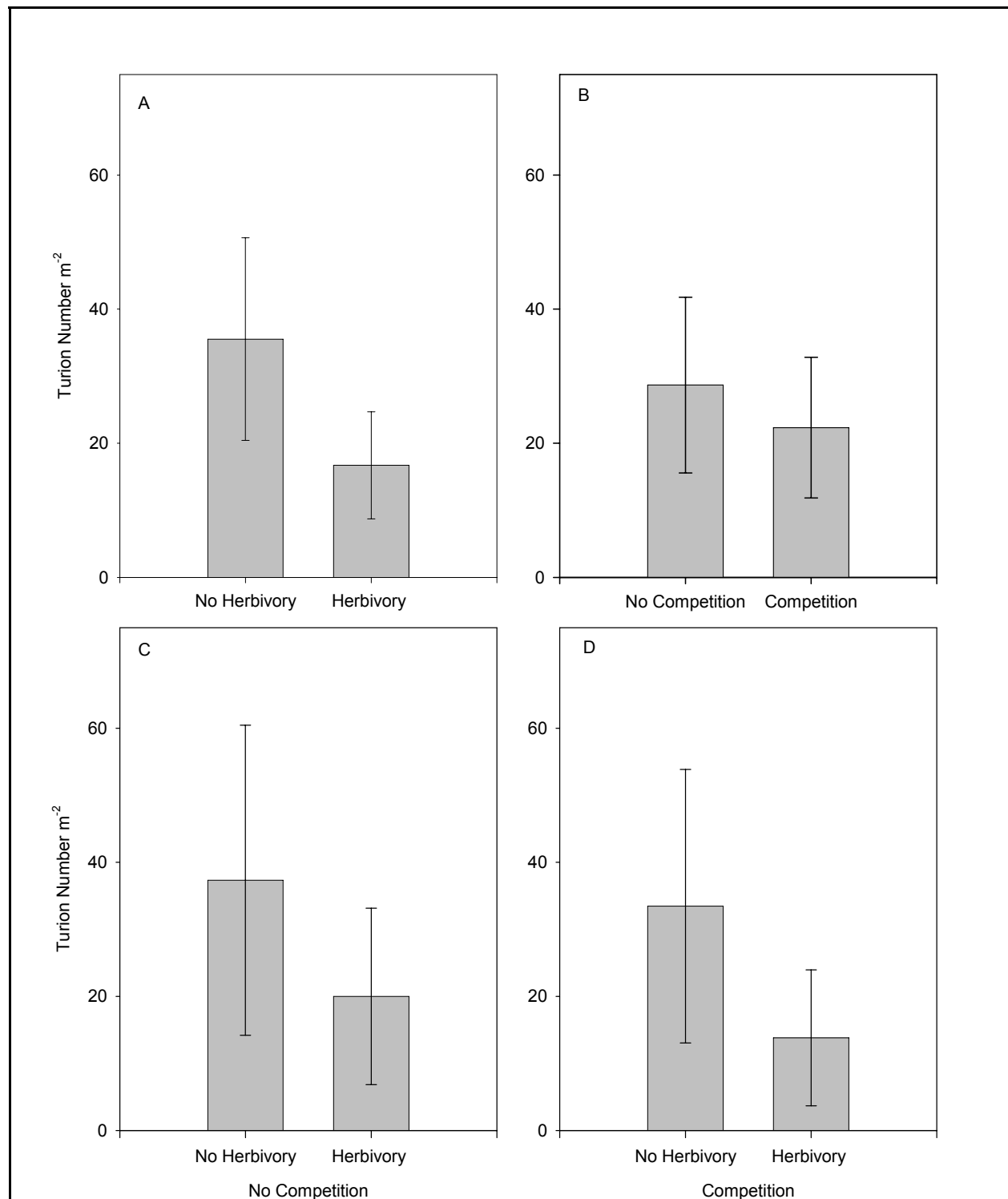


Figure 3. Turion numbers  $m^{-2}$  for hydrilla ponds with and without herbivory ( $p=.02427$ ,  $F=5.21$ , Figure 3A), ponds with and without competition ( $p=.45163$ ,  $F=0.57$ , Figure 3B), and hydrilla interaction between all treatments ( $p=.89008$ ,  $F=0.019$ , Figure 3C,D). Bars represent 95-percent confidence intervals.

Significant differences in tuber numbers were observed for both herbivory (Figure 4A) and competition (Figure 4B). A 39-percent reduction in tuber numbers was noted in those ponds containing sustained insect herbivory (Figure 4A). Doyle et al. (2002) likewise found tuber numbers were reduced by 55 percent under high levels of herbivory.

Similar reductions were noted for competition effects. Ponds with no competition averaged  $>130$  tubers/m<sup>2</sup> versus only about 40 tubers/m<sup>2</sup> observed in the competition ponds (Figure 4B). Although not significantly different, individual tuber DW was also found to be greater for the no competition ponds at 0.192 g DW versus 0.145 g DW for tubers produced in the competition ponds; a reduction of almost 25 percent. One possible explanation for the differences in tuber production is that although the competition ponds only had good native plant coverage the first growing season (Owens, pers. obser.), the competition for resources between hydrilla and the native plants may have impacted hydrilla tuber production for the first growing season. Netherland (1999) found that competition limited tuber production and reduced rootcrown density. It appeared that native plants were able to actively compete for resources, thus limiting the ability of hydrilla to produce as many tubers.

In conclusion, herbivory over this 2-year study period significantly reduced hydrilla biomass, tuber production, and tuber dry weight. Competition significantly reduced tuber production. By increasing stress factors on hydrilla populations through resource competition and leaf mining, all aspects of the life cycle of hydrilla can be negatively impacted. Results from this study in addition to earlier research (Doyle et al. 2002, Doyle et al. 2005, Grodowitz et al. 2003) indicate that establishment of leaf-mining flies in synchronization with native plant competition can result in decreased hydrilla biomass and reduction in tuber and turion production.

**ACKNOWLEDGEMENTS:** This research was conducted under the U.S. Army Corps of Engineers Aquatic Plant Control Research Program, U.S. Army Engineer Research and Development Center. Permission to publish this information was granted by the Chief of Engineers. We would like to thank Robin Bare and Christie Snell for technical and field assistance for this project and Dr. Judy Shearer and LeeAnn Glomski for review of this technical note.

**POINTS OF CONTACT:** This technical note was written by Dr. Michael Grodowitz, Ms. Chetta Owens, Dr. Michael Smart, and Ms. Julie Nachtrieb, Environmental Laboratory (EL), U.S. Army Engineer Research and Development Center (ERDC). For additional information, contact the manager of the Aquatic Plant Control Research Program, Mr. Robert C. Gunkel (601-634-3722, [Robert.C.Gunkel@erdc.usace.army.mil](mailto:Robert.C.Gunkel@erdc.usace.army.mil)). This technical note should be cited as follows:

Grodowitz, M. J., C. S. Owens, R. M. Smart, and J. G. Nachtrieb. 2007. *Impact of herbivory and plant competition on the growth of hydrilla in small ponds*. ERDC/TN APCRP-BC-08, Vicksburg, MS: U.S. Army Engineer Research and Development Center.



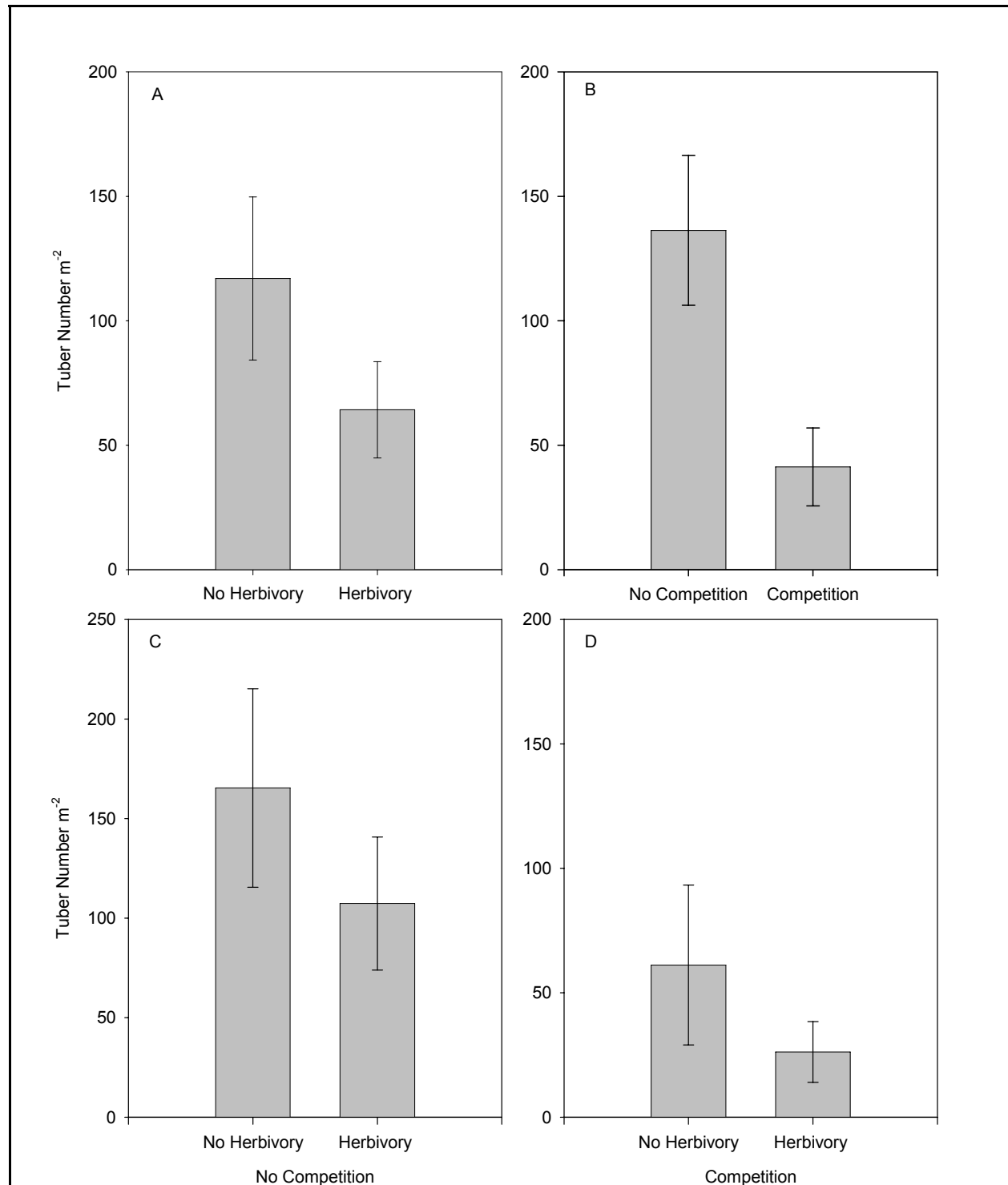


Figure 4. Tuber numbers  $m^{-2}$  for hydrilla ponds with and without herbivory ( $p=.00510$ ,  $F=8.15$ , Figure 4A) ponds with and without competition ( $p=.00000$ ,  $F=31.45$ , Figure 4B), and hydrilla interaction between all treatments ( $p=.48874$ ,  $F=0.48$ , Figure 4C,D). Bars represent 95-percent confidence intervals.

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